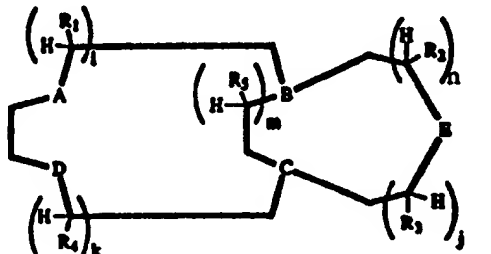




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 49/00, C07D 245/00, 487/00	A1	(11) International Publication Number: WO 95/19185 (43) International Publication Date: 20 July 1995 (20.07.95)
(21) International Application Number: PCT/US95/00634 (22) International Filing Date: 13 January 1995 (13.01.95) (30) Priority Data: 08/182,243 14 January 1994 (14.01.94) US (71) Applicant: MALLINCKRODT MEDICAL, INC. [US/US]; 675 McDonnell Boulevard, P.O. Box 5840, St. Louis, MO 63134 (US). (72) Inventors: WALLACE, Rebecca, A.; 1444 Sunnyside Lane, Manchester, MO 63021 (US). DUNN, T., Jeffrey; 9505 Byrnesville Road, Cedar Hill, MO 63016 (US). MOORE, Dennis, A.; 111 Barto Drive, Florissant, MO 63031 (US). (74) Agents: STIERWALT, Brian, K. et al.; Mallinckrodt Medical, Inc., 675 McDonnell Boulevard, P.O. Box 5840, St. Louis, MO 63134 (US).		(81) Designated States: AU, BR, CA, CZ, FI, HU, JP, MX, NO, PL, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i>
(54) Title: FUNCTIONALIZED AZA-MACROBICYCLIC LIGANDS FOR IMAGING APPLICATIONS (57) Abstract <p>The present invention provides new and structurally diverse compositions comprising compounds of general formula (I), wherein A is N-G or P-G; B is N or P; C is N or P; D is N-G or P-G; E is N-G, P-G or C(R₆)-[CH(R₇)]_q-X; G is -[CH(R₈)]_i-X; X is -CO₂CH₃, -OPO₃H₂, -PO₃H₂, -SO₃H, -SH, -OH, or -CONHOH; R₁, R₂, R₃, R₄, R₅, R₆, R₇ and R₈ can be the same or different and are hydrogen, C₁-C₈ alkyl, or C₆-C₁₀ aryl, optionally substituted by one or more hydroxy, C₁-C₈ alkyl, C₁-C₈ hydroxyalkyl, C₁-C₈ alkoxy, C₆-C₁₀ aryl, C₆-C₁₀ hydroxyaryl, C₆-C₁₀ aryloxy, -CO₂R₇, -CONR₁₀R₁₁, or -NR₁₀R₁₁ groups; R₉, R₁₀ and R₁₁ may be the same or different and are selected from the group consisting of hydrogen C₁-C₈ alkyl, C₁-C₈ hydroxyalkyl and C₁-C₈ alkoxyalkyl, R₁₀ and R₁₁ may form a 5- or 6-membered carbocyclic ring optionally containing singularly or in combination nitrogen, oxygen or sulfur; i, j, k, l, m, n and q may be the same or different and are zero to about 5. Methods for imaging using compositions of the invention are also provided.</p> <div style="text-align: center;">  </div>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

FUNCTIONALIZED AZA-MACROBICYCLIC LIGANDS FOR IMAGING APPLICATIONS

FIELD OF THE INVENTION

5 This invention relates to magnetic resonance imaging (MRI),
X-ray imaging, and radiopharmaceuticals. More particularly the
invention relates to methods and compositions for enhancing MRI,
X-ray imaging, and radiopharmaceuticals.

10 BACKGROUND OF THE INVENTION

The use of contrast agents in diagnostic medicine is rapidly growing. In X-ray diagnostics, for example, increased contrast of internal organs, such as the kidneys, the urinary tract, the digestive tract, the vascular system of the heart (angiography), and so forth is obtained by administering a contrast agent which is substantially radiopaque. In conventional proton MRI diagnostics, increased contrast of internal organs and tissues may be obtained by administering compositions containing paramagnetic metal species which increase the relaxivity of surrounding protons. In ultrasound diagnostics, improved contrast is obtained by administering compositions having acoustic impedances different than that of blood and other tissues.

The recently developed technique of MRI encompasses the detection of certain atomic nuclei utilizing magnetic fields and radio-frequency radiation. It is similar in some respects to X-ray computed tomography (CT) in providing a cross-sectional display of the body organ anatomy with

excellent resolution of soft tissue detail. As currently used, the images produced constitute a map of the proton density distribution, the relaxation times, or both, in organs and tissues. The technique of MRI is advantageously non-
5 invasive as it avoids the use of ionizing radiation.

While the phenomenon of NMR was discovered in 1945, it is only recently that it has found application as a means of mapping the internal structure of the body as a result of
10 the original suggestion of Lauterbur (Nature, 242, 190-191 [1973]). The fundamental lack of any known hazard associated with the level of the magnetic and radio-frequency fields that are employed renders it possible to make repeated scans on vulnerable individuals. In addition to standard scan planes
15 (axial, coronal, and sagittal), oblique scan planes can also be selected.

With an MRI experiment, the nuclei under study in a sample (e.g. protons) are irradiated with the appropriate
20 radio-frequency (RF) energy in a highly uniform magnetic field. These nuclei, as they relax, subsequently emit RF at a sharp resonance frequency. The resonance frequency of the nuclei depends on the applied magnetic field.

According to known principles, nuclei with
25 appropriate spin, when placed in an applied magnetic field (B, expressed generally in units of gauss or Tesla [10^4 gauss]) align in the direction of the field. In the case of protons, these nuclei precess at a frequency, f , of 42.6 MHz, at a field strength of 1 Tesla. At this frequency, an RF pulse of
30 radiation will excite the nuclei and can be considered to tip the net magnetization out of the field direction, the extent of this rotation being determined by the pulse duration and energy. After the RF pulse, the nuclei "relax" or return to equilibrium with the magnetic field, emitting radiation at the
35 resonant frequency. The decay of the emitted radiation is

characterized by two relaxation times, i.e., T_1 , the spin-lattice relaxation time or longitudinal relaxation time, that is, the time taken by the nuclei to return to equilibrium along the direction of the externally applied magnetic field, and T_2 , the spin-spin relaxation time associated with the dephasing of the initially coherent precession of individual proton spins. These relaxation times have been established for various fluids, organs and tissues in different species of mammals.

In MRI, scanning planes and slice thicknesses can be selected. This selection permits high quality transverse, coronal and sagittal images to be obtained directly. The absence of any moving parts in MRI equipment promotes high reliability. It is believed that MRI has a greater potential than CT for the selective examination of tissue characteristics in view of the fact that in CT, X-ray attenuation coefficients alone determine image contrast, whereas at least five separate variables (T_1 , T_2 , proton density, pulse sequence and flow) may contribute to the MRI signal.

By reason of its sensitivity to subtle physico-chemical differences between organs and/or tissues, it is believed that MRI may be capable of differentiating different tissue types and in detecting diseases which induce physicochemical changes that may not be detected by X-ray or CT which are only sensitive to differences in the electron density of tissue.

As noted above, two of the principal imaging parameters are the relaxation times, T_1 and T_2 . For protons (or other appropriate nuclei), these relaxation times are influenced by the environment of the nuclei, (e.g., viscosity, temperature, and the like). These two relaxation

phenomena are essentially mechanisms whereby the initially imparted radio-frequency energy is dissipated to the surrounding environment. The rate of this energy loss or relaxation can be influenced by certain other nuclei which are paramagnetic. Chemical compounds incorporating these paramagnetic nuclei may substantially alter the T_1 and T_2 values for nearby protons. The extent of the paramagnetic effect of a given chemical compound is a function of the environment.

In general, paramagnetic species such as ions of elements with atomic numbers of 22 to 29, 42 to 44 and 58 to 70 have been found effective as MRI image contrasting agents. Examples of suitable ions include chromium(III), manganese(II), manganese(III), iron(II), iron(III), cobalt(II), nickel(II), copper(II), praseodymium(III), neodymium(III), samarium(III), and ytterbium(III). Because of their very strong magnetic moments, gadolinium(III), terbium(III), dysprosium(III), holmium(III) and erbium(III) are preferred. Gadolinium(III) ions have been particularly preferred as MRI contrasting agents.

Typically, paramagnetic ions have been administered in the form of complexes with organic complexing agents. Such complexes provide the paramagnetic ions in a soluble, non-toxic form, and facilitate their rapid clearance from the body following the imaging procedure. Gries et al., U.S. Patent 4,647,447, disclose complexes of various paramagnetic ions with conventional aminocarboxylic acid complexing agents. A preferred complex disclosed by Gries et al. is the complex of gadolinium(III) with diethylenetriamine-pentaacetic acid ("DTPA").

Paramagnetic ions, such as gadolinium(III), have been found to form strong complexes with DTPA, ethylenediamine-tetraacetic acid ("EDTA"), and with tetraazacyclododecane- N,N',N'',N''' -tetraacetic acid ("DOTA").

These complexes do not dissociate substantially in physiological aqueous fluids. The gadolinium complex of DTPA has a net charge of -2, whereas the gadolinium complex of EDTA or DOTA has a net charge of -1, and both are generally administered as soluble salts. Typical salts are sodium and N-methylglucamine. The administration of salts is attended by certain disadvantages. These salts can raise the in vivo ion concentration and cause localized disturbances in osmolality, which in turn, can lead to edema and other undesirable reactions.

Efforts have been made to design new ionic and neutral paramagnetic metal complexes which avoid or minimize the above mentioned disadvantages. In general, this goal can be achieved by converting one or more of the free carboxylic acid groups of the complexing agent to neutral, non-ionizable groups. For example, S.C. Quay, in U.S. Patents 4,687,658 and 4,687,659, discloses alkylester and alkylamide derivatives, respectively, of DTPA complexes. Similarly, published Dean et al., U.S. Patent Number 4,826,673 discloses mono- and polyhydroxyalkylamide derivatives of DTPA and their use as complexing agents for paramagnetic ions. It can also be achieved by covalent attachment of organic cations to the complexing agent in such a manner that the sum of positive and negative charges in the resulting metal complex is zero.

The nature of additional substituents in the complexing agent can have a significant impact on tissue specificity. Hydrophilic complexes tend to concentrate in the interstitial fluids, whereas lipophilic complexes tend to associate with cells. Thus, differences in hydrophilicity can lead to different applications of the compounds. See, for example, Weinmann et al., AJR, 142, 679 (Mar. 1984) and Brasch, et al., AJR, 142, 625 (Mar. 1984).

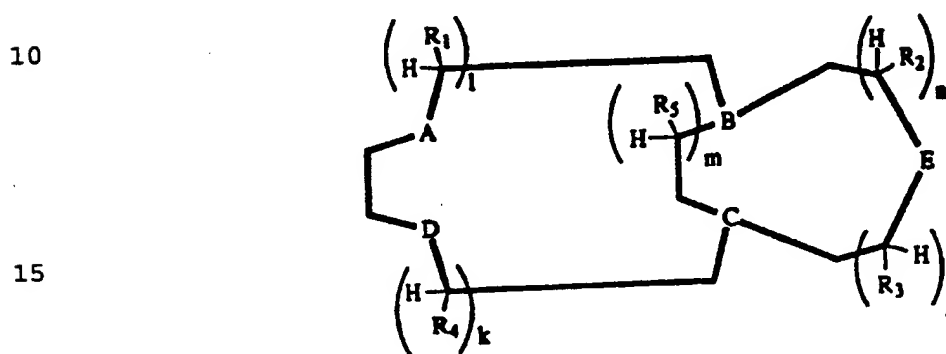
Finally, toxicity of paramagnetic metal complexes is greatly affected by the nature of the complexing agents. In vivo release of free metal ions from the complex is a major cause of toxicity. Four principal factors are important in the design of chelates for making paramagnetic metal complexes that are highly stable in vivo and less toxic. The first three factors are thermodynamic in nature whereas the fourth involves chelate kinetics. The first factor is the thermodynamic stability constant of the metal-ligand. The thermodynamic stability constant indicates the affinity that the totally unprotonated ligand has for a metal. The second factor is the conditional stability constant which takes into account the pH and is important when considering stability under physiological pH. The selectivity of the ligand for the paramagnetic metal over other endogenous metal ions such as zinc, iron, magnesium and calcium is the third factor. In addition to the three thermodynamic considerations, complexes with structural features that make in vivo transmetallation reactions much slower than their clearance rates would be predicted to have low toxicities. Therefore, in vivo reaction kinetics are a major factor in the design of stable complexes. See, for example, Cacheris et al., Magnetic Resonance Imaging, 8:467 (1990) and Oksendal, et al., JMRI, 3:157 (1993).

A need continues to exist for new and structurally diverse compounds for use as imaging agents and radiopharmaceuticals. There is a further need to develop highly stable complexes with good relaxivity and osmolar characteristics.

SUMMARY OF THE INVENTION

5

The present invention provides new and structurally diverse compositions comprising compounds of the general formula:



20 Wherein A is N-G or P-G; B is N or P; C is N or P; D is N-G or P-G; E is N-G, P-G or C(R₅)-[CH(R₇)]_q-X; G is -[CH(R₈)]₁-X; X is -CO₂H, -OPO₃H₂, -PO₃H₂, -SO₃H, -SH, -OH, or -CONHOH; R₁, R₂, R₃, R₄, R₅, R₆, R₇ and R₈ can be the same or different and are hydrogen, C₁-C₈ alkyl, or C₆-C₁₀ aryl, optionally

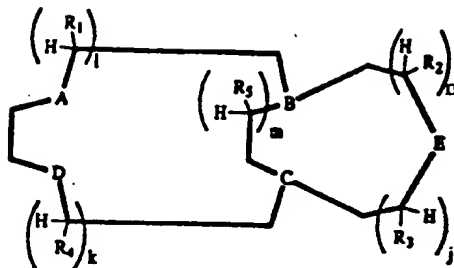
25 substituted by one or more hydroxy, C₁-C₈ alkyl, C₁-C₈ hydroxyalkyl, C₁-C₈ alkoxy, C₆-C₁₀ aryl, C₆-C₁₀ hydroxyaryl, C₆-C₁₀ aryloxy, -CO₂R₇, -CONR₁₀R₁₁, or -NR₁₀R₁₁ groups; R₉, R₁₀, and R₁₁ may be the same or different and are selected from the group consisting of hydrogen, C₁-C₈ alkyl, C₁-C₈ hydroxyalkyl and C₁-C₈ alkoxyalkyl;

30 R₁₀ and R₁₁ may form a 5 or 6 membered carbocyclic ring optionally containing singularly or in combination nitrogen, oxygen or sulfur; i, j, k, l, m, n and q may be the same or different and are zero to about 5.

Also provided are compositions comprising complexes of the compounds with metal ions of the general formula

35

5



- 10 Wherein A is N-G or P-G; B is N or P; C is N or P; D is
N-G or P-G; E is N-G, P-G or C(R₆)-[CH(R₇)]_q-Y; G is -[CH(R₈)]_l-
Y; Y is -CO₂M, -OPO₃HM, -PO₃HM, -SO₃M, -SM, -OM, or -CONHOM; R₁,
R₂,
R₃, R₄, R₅, R₆, R₇, and R₈ can be the same or different and are
15 hydrogen, C₁-C₈ alkyl, or C₆-C₈ alkyl, C₆-C₁₀ aryl, optionally
substituted by one or more hydroxy, C₁-C₈ alkoxy, C₁-C₈
hydroxyalkyl, C₁-C₈ alkoxy, C₆-C₁₀ hydroxyaryl, C₆-C₁₀ aryloxy, -
CO₂R₉, -CONR₁₀R₁₁, or NR₁₀P₁₁ groups, R₉, R₁₀, and R₁₁ may be the
20 same or different and are selected from the group consisting
of hydrogen, C₁-C₈ alkyl alkyl, C₁-C₈ hydroxyalkyl and C₁-C₈
alkoxyalkyl; R₁₀ and R₁₁ may form a 5 or 6 membered carbocyclic
ring optionally containing singularly or in combination
nitrogen, oxygen or sulfur; i, j, k, l, m, n and q may be the
25 same or different and are zero to about 5; and M is a metal
ion equivalent and/or a physiologically acceptable cation of
an organic base.

30 Compositions comprising the above formulas wherein M is a
radioactive metal ion, a paramagnetic ion, or a metal ion
capable of absorbing x-rays are also provided for use as
radiopharmaceuticals, magnetic resonance imaging, and x-ray
contrast agents, respectively.

35 Diagnostic compositions comprising the compounds of the
invention are also provided. Methods of performing diagnostic

procedures with compositions of the invention are also disclosed. The methods comprise administering to a patient an effective amount of the compositions of the invention and optionally subjecting the patient to an imaging procedure of imaging.

DETAILED DESCRIPTION

The compositions of the invention are suitable for use with a variety of modalities including x-rays, magnetic resonance imaging and radiopharmaceuticals.

The functionality of the R groups of the compositions of the invention afford the additional capability of derivatization to biomolecules and synthetic polymers.

Biomolecule refers to all natural and synthetic molecules that play a role in biological systems. Biomolecules include hormones, amino acids, peptides, peptidomimetics, proteins, deoxyribonucleic acid (DNA) ribonucleic acid (RNA), lipids, albumins, polyclonal antibodies, receptor molecules, receptor binding molecules, monoclonal antibodies and aptamers.

Specific examples of biomolecules include insulins, prostaglandins, growth factors, liposomes and nucleic acid probes. Examples of synthetic polymers include polylysine, arborols, dendrimers, and cyclodextrins. The advantages of using biomolecules include enhanced tissue targeting through specificity and delivery. Coupling of the chelating moieties to biomolecules can be accomplished by several known methods (e.g., Krejcarek and Tucker Biochem. Biophys. Res. Comm. 30, 581 (1977); Hnatowich, et al. Science, 220, 613 (1983). For example, a reactive moiety present in one of the R groups is coupled with a second reactive group located on the biomolecule. Typically, a nucleophilic group is reacted with an electrophilic group to form a covalent bond between the biomolecule and the chelate. Examples of nucleophilic groups include amines, anilines, alcohols, phenols, thiols and

hydrazines. Electrophilic group examples include halides, disulfides, epoxides, maleimides, acid chlorides, anhydrides, mixed anhydrides, activated esters, imidates, isocyanates and isothiocyanates. And finally, the compositions of the invention should provide the additional advantage of being kinetically inert.

Examples of suitable alkyl groups for use with the invention include methyl, ethyl, propyl, isopropyl, butyl, cyclohexyl, heptyl and octyl. Suitable alkoxy groups include methoxy, ethoxy, propoxy, butoxy, pentoxy, hexoxy, heptoxy and octoxy. Hydroxyalkyl groups suitable for use with the invention include both mono and poly hydroxyalkyls such as hydroxyethyl, 2-hydroxypropyl, 2,3-dihydroxypropyl, 2,3,4-trihydroxybutyl, tris(hydroxymethyl)methyl and 2-hydroxy-1-hydroxymethyl-ethyl. Suitable alkoxyalkyl groups include methoxymethyl, 2,3-dimethoxypropyl, tris(methoxymethyl)methyl, and 2-methoxy-1-methoxymethyl-ethyl.

Examples of suitable compounds of the invention are 4, 7, 13-tris(carboxymethyl)-1, 4, 7, 10, 13-pentaazabicyclo[8.5.2]pentadecane; 4, 7, 12-tris(carboxymethyl)-12-methyl-1, 4, 7, 10-tetraazabicyclo[8.3.2]-pentadecane; 5, 8, 15-tris(carboxymethyl)-1, 5, 8, 12, 15-pentaazabicyclo[10.5.2]nonadecane; and 4, 7, 13-tris(mercaptoethyl)-1, 4, 7, 10, 13-pentaazabicyclo[8.5.2]pentadecane.

Complexes of the novel ligands or compounds of the invention with one or more central metal ions or metal ion equivalents such as paramagnetic metals praseodymium(III), neodymium(III), samarium(III), ytterbium(III) terbium(III), dysprosium(III), holmium(III), erbium(III), iron(II), iron(III), manganese(II), manganese(III), gadolinium(III), chromium(III), cobalt(II) and nickel(II) are useful for enhancing magnetic resonance images. While such metal ions

are themselves paramagnetic in nature and capable of altering the magnetic resonance signal characteristics of body tissues, organs or fluids, they may exhibit significant toxicity when administered in the form of ionic salts. However, novel
5 complexes of the invention are relatively or substantially nontoxic and therefore useful for enhancing magnetic resonance images by favorably altering relaxation times T_1 and T_2 and affording improved contrast between normal and diseased tissues or organs.

10

The preferred complexes of the invention are those formed from the above ligands and iron(II), iron(III), manganese(II), manganese(III) and gadolinium(III) as the central metal ion or ions. Depending upon the particular ligand employed and the
15 particular central metal ion used, the complexes formed may be neutral, ionic, cationic, or zwitterionic in nature, or they may be negatively charged. The neutral complexes are generally preferred and generally appear to exhibit relatively lower toxicity as compared to ionic or negatively charged
20 complexes. The negatively charged complexes formed by the ligands and central metal ions enumerated above may be further complexed with one or more cations of an inorganic or organic base which are physiologically tolerated. Examples of cations for further complexing include sodium, potassium, calcium, and
25 salts of N-methylglucamine, and diethanolamine.

Examples of preferred compounds of the invention and one or more central metal ions (i.e., complexes) include
4, 7, 13-tris(carboxymethyl)-1, 4, 7, 10, 13-
30 pentaazabicyclo[8.5.2]pentadecane, gadolinium (III) complex;
4, 7, 12-tris(carboxymethyl)-12-methyl-1, 4, 7, 10-
tetraazabicyclo[8.3.2]-pentadecane, dysprosium(III) complex;
5, 8, 15-tris(carboxymethyl)-1, 5, 8, 12, 15-
pentaazabicyclo[10.5.2]nonadecane, gadolinium(III) complex;
35 and 4, 7, 13-tris(mercaptoethyl)-1, 4, 7, 10, 13-

pentaazabicyclo[8.5.2]pentadecane, iron(III) complex.

In addition to their utility in magnetic resonance
5 imaging procedures, the compositions of the invention can also
be employed for delivery of either radiopharmaceuticals or
heavy metals for x-ray contrast into the body. For use in
diagnostic and therapeutic radiopharmaceuticals the complexed
metal ion must be radioactive. Radioisotopes of the elements
10 technetium, rhenium, indium, gallium, copper, yttrium,
samarium and holmium are suitable. For use as X-ray contrast
applications the complexed metal ion must be able to absorb
adequate amounts of the X-rays. These metal ions are
generally referred to as radioopaque. Suitable elements for
15 use as the radioopaque metal ion include lead, bismuth,
gadolinium, dysprosium, holmium and praseodymium.

Examples of preferred compounds for radiopharmaceuticals
are 4, 7, 13-tris(carboxymethyl)-1, 4, 7, 10, 13-
20 pentaazabicyclo[8.5.2]pentadecane, yttrium(III) complex; 4, 7,
12-tris(carboxymethyl)-12-methyl-1, 4, 7, 10-
tetraazabicyclo[8.3.2]-pentadecane, indium(III) complex; 5, 8,
15-tris(carboxymethyl)-1, 5, 8, 12, 15-
pentaazabicyclo[10.5.2]nonadecane, gallium(III) complex; and
25 4, 7, 13-tris(mercaptoethyl)-1, 4, 7, 10, 13-
pentaazabicyclo[8.5.2]pentadecane, indium(III) complex.

Examples of preferred compounds for x-ray contrast are
4, 7, 13-tris(carboxymethyl)-1, 4, 7, 10, 13-
30 pentaazabicyclo[8.5.2]pentadecane, holmium(III) complex; 4, 7,
12-tris(carboxymethyl)-12-methyl-1, 4, 7, 10-
tetraazabicyclo[8.3.2]-pentadecane, gadolinium(III) complex;
5, 8, 15-tris(carboxymethyl)-1, 5, 8, 12, 15-
pentaazabicyclo[10.5.2]nonadecane, dysprosium(III) complex;
35 and 4, 7, 13-tris(mercaptoethyl)-1, 4, 7, 10, 13-

pentaazabicyclo[8.5.2]pentadecane, bismuth (III) complex.

The compositions of the invention can be formulated into therapeutic or diagnostic compositions for enteral or parenteral administration. These compositions contain an effective amount of the paramagnetic ion complex along with conventional pharmaceutical carriers and excipients appropriate for the type of administration contemplated. For example, parenteral formulations advantageously contain a sterile aqueous solution or suspension of from about 0.05 to about 1.0M of a paramagnetic ion complex according to this invention. Parenteral compositions may be injected directly or mixed with a large volume parenteral composition for systemic administration. Preferred parenteral formulations have a concentration of paramagnetic ion complex of about 0.1M to about 0.5M. Such solutions also may contain pharmaceutically acceptable buffers and, optionally, electrolytes such as sodium chloride. The compositions may advantageously contain a slight excess (e.g., from about 0.01 to about 15.0 mole % excess) of a complexing agent or its complex with a physiologically acceptable, non-toxic cation. Such physiologically acceptable, non-toxic cations include calcium ions, magnesium ions, copper ions, zinc ions, salts of n-methylglucamine and diethanolamine, and the like. Generally, calcium ions are preferred.

Formulations for enteral administration may vary widely, as is well-known in the art. In general, such formulations are liquids which include an effective amount of the paramagnetic ion complex in aqueous solution or suspension. Such enteral compositions may optionally include buffers, surfactants, thixotropic agents, and the like. Compositions for oral administration may also contain flavoring agents and other ingredients for enhancing their organoleptic qualities.

The diagnostic compositions are administered in doses effective to achieve the desired enhancement of the image. Such doses may vary widely, depending upon the particular paramagnetic ion complex employed, the organs or tissues which are the subject of the imaging procedure, the imaging procedure, the imaging equipment being used, and the like. In general, parenteral dosages will range from about 0.001 to about 1.0 mMol of paramagnetic ion complex per kg of patient body weight. Preferred parenteral dosages range from about 0.01 to about 0.5 mMol of paramagnetic ion complex per kg of patient body weight. Enteral dosages generally range from about 0.5 to about 100 mMol, preferably from about 1.0 to about 10 mMol, more preferably from about 1.0 to about 10.0 mMol of paramagnetic ion complex per kg of patient body weight.

The diagnostic compositions of the invention are used in the conventional manner. The compositions may be administered to a patient, typically a warm-blooded animal, either systemically or locally to the organ or tissue to be imaged, and the patient then subjected to the imaging procedure. Protocols for imaging and instrument procedures are found in texts such as Stark, D.D.; Bradley, W.G. *Magnetic Resonance Imaging*; Mosby Year Book: St. Louis, MO, 1992.

Radiopharmaceutical Imaging Procedures are found in Fred A. Mettler, Jr., M.D., M.P.H., Milton J. Guiberteau, M.D., Essentials of Nuclear Medicine Imaging, Grune and Stratton, Inc., New York, NY 1983) and E. Edmund Kim, M.S., M.D. and Thomas P. Haynie, M.D., (MacMillan Publishing Co. Inc., New York, NY 1987).

X-ray contrast Imaging Procedures are found in Albert A. Moss, M.D., Gordon Gamsu, M.D., and Harry K. Genant, M.D., Computed Tomography of the Body, (W.B. Saunders Company,

Philadelphia, Pennsylvania 1992) and M. Sovak, Editor,
Radiocontrast Agents, (Springer-Verlag, Berlin 1984).

5 The following examples illustrate the specific
embodiments of the invention described in this document. As
would be apparent to skilled artisans, various changes and
modifications are possible and are contemplated within the
scope of the invention described.

10

EXAMPLES

Example 1

15

Synthesis of 5,8,15-Tris(carboxymethyl)-1,5,8,12,15-
pentaazabicyclo-[10.5.2]nona-decane

20

To a stirred solution consisting of 10.0g (0.166mole, 11.2mL)
ethylenediamine, 50.4g (0.498mole, 69.4mL) triethylamine and
250mL dichloromethane is added, dropwise, a solution of 73.3g
(0.365mole) 2-trimethylsilylethylsulfonyl chloride in 150mL
dichloromethane. When the addition is complete, the mixture
is placed in separatory funnel and washed with 2x500mL 1.0N
25 HCl, and 2x500mL 1.0N NaOH. The organic layer is collected
and dried with MgSO₄. After the drying agent is removed by
filtration, the solvent is evaporated and the dry white solid
remaining is crystallized from boiling methanol containing 10%
water. The clear colorless crystals which form are dried in
30 air. Yield of 1,4-bis(trimethylsilylethylsulfonyl)-1,4-
diazabutane; 43.2g (67.0% based on ethylenediamine). Melting
point 166-7C. Identity and purity of the product is confirmed
by ¹H and ¹³C nmr, and elemental analysis.

35

A solution containing 18.2g (0.173mole) diethanolamine and

150mL (1.08mole, 108.9g) triethylamine in 500mL dichloromethane is cooled in an ice-water bath. To this solution is added a solution containing 108.6g (0.570mole) p-toluene-sulfonyl chloride in 200mL dichloromethane. The rate
5 of addition is such that the temperature of the reaction mixture does not exceed 5C. When the addition is complete, the mixture is stored in 2L flask fitted with a CaCl₂ drying tube in a OC refrigerator overnight. The cold solution is filtered to remove the large amount of crystals which form
10 (HNET₃+Cl⁻) and concentrated by evaporation in vacuo to a thick oil. The oil is shaken with 1000g ice and water and the precipitate which forms is collected by filtration. The solid is dissolved in 300mL fresh dichloromethane and washed with 3x150mL 1.0N HCl. The organic layer is collected and dried
15 with MgSO₄. After removing the drying agent by filtration the solvent is removed by evaporation and the oil which forms is dissolved in a minimum of boiling methanol/ethyl acetate (20:1), ca. 250mL. Upon cooling, crystals of 1,4,7-tris(p-toluenesulfonyl)-4-aza-1,7-dioxoheptane are formed. Yield
20 83.0g (98.2% based on diethanolamine). Identity and purity of the product is confirmed by ¹H and ¹³C nmr, and elemental analysis.

To a slurry containing 9.06g (60% dispersion in mineral oil,
25 0.226mole) sodium hydride in 250mL dry dmf is added, dropwise, a solution of 40g (0.103mole) 1,4-bis(trimethylsilylethylsulfonyl)-1,4-diazabutane, in 250mL dry dmf. When the addition is complete the mixture is briefly heated to 60C and allowed to cool to room temperature. When
30 cool, the mixture is filtered to remove unreacted NaH and the solution returned to a reaction vessel. The solution is heated, under dry air, to 85C and a solution containing 64.3g (0.113mole) 1,4,7-tris(p-toluenesulfonyl)-4-aza-1,7-dioxoheptane, in 200mL dry dmf is added. When the addition is
35 complete, the mixture is allowed to stir overnight. After

cooling the mixture to room temperature, the solvent is removed in vacuo, and the pasty solid remaining is treated with 500g ice. The resulting precipitate is collected by filtration and washed with distilled water until the pH of the filtrate is neutral. The solid is pressed dry to remove most of the water present and dissolved in a minimum amount of boiling methanol and acetone (20:1). The hot solution is quickly filtered to remove any particulates and the solution allowed to stand. Upon cooling, crystals of 1,4-bis(2-trimethylsilylethylsulfonyl)-7-(p-toluenesulfonyl)-1,4,7-triazacyclononane are deposited. Yield 41.1g (65.0%) Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental analysis.

A slurry consisting of 40.0g (65.1mmoles) 1,4-bis(2-trimethylsilylethyl-sulfonyl)-7-(p-toluenesulfonyl)-1,4,7-triazacyclononane and 29.7g (195mmoles) CsF in 100mL dry dmf is refluxed overnight. After cooling to room temperature 50mL methanol is added and the mixture evaporated in vacuo. The residue is diluted with 50mL diethyl ether, heated briefly to reflux, filtered and allowed to stand. Upon cooling, crystals of p-toluenesulfonyl-1,4,7-triazacyclononane are deposited. Yield 14.9g (81%). Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental analysis.

To a flask containing 14.0g (4.94mmoles) 1-p-toluenesulfonyl-1,4,7-triazacyclononane is added 50.0 mL acrylonitrile. The mixture is refluxed briefly, cooled and allowed to stir overnight. The solvent is removed by evaporation, in vacuo. The oil is dissolved in 50:50 1.0N HCl:ethanol and slurried with 2.00g (8.81mmoles) PtO_2 . The mixture is shaken overnight at 60psi H_2 . Note: the reaction vessel will need to be refilled several times with H_2 as the reaction proceeds. After the unreacted H_2 is vented, the mixture is filtered to remove the catalyst. The filtrate is evaporated and the mixture

treated with methanol containing 0.868g (21.7mmoles) sodium hydroxide. The mixture is evaporated and the residue slurried with 200mL dichloromethane. This mixture is treated with MgSO_4 . After the drying agent is removed by filtration, the
5 clear colorless solution is evaporated leaving 1,4-bis(3-aminopropyl)-7-(p-toluenesulfonyl)-1,4,7-triazacyclo-nonane as a thick pale oil. Yield 14.7g (75%). Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental analysis.

10

A solution containing 14.0g (35.2mmoles) 1,4-bis(3-aminopropyl)-7-(p-toluenesulfonyl)-1,4,7-triazacyclononane in 50mL concentrated sulfuric acid is allowed to stir for three days. The mixture is cooled in an ice bath and poured very
15 carefully into 500mL cold (0C) diethyl ether. The mother liquid is decanted from the resulting oil. The oil is treated with a solution consisting of 8.5g (212mmoles) sodium hydroxide in 100mL methanol. The alcohol is evaporated and the residue slurried with 200mL dichloromethane. The slurry
20 is treated with MgSO_4 . After the drying agent is removed by filtration the solution is evaporated giving 1,4-bis(3-aminopropyl)-1,4,7-triazacyclononane as a pale oil. Yield 7.02g (85%). Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental analysis.

25

To a solution containing 7.00g (28.8mmoles) 1,4-bis(3-aminopropyl)-1,4,7-triazacyclononane in 95% ethanol is added 5.39g (31.6mmoles) copper (II) chloride dihydrate. The mixture is heated to reflux for two hours, then allowed to
30 cool. Then solvent is removed by evaporation in vacuo and the residue redissolved in 95% aqueous methanol. To this solution is added 5.4mL (37.4mmoles) 40% aqueous glyoxal. The mixture is refluxed overnight. After removing the solvent by evaporation, the residue is dissolved in 1L 90% aqueous
35 methanol and treated with 3.26g (86.3mmoles) NaBH_4 and refluxed

for one hour. After cooling to room temperature the mixture is evaporated to dryness, redissolved in 200mL fresh methanol and evaporated again. The residue is dissolved in 100mL water and the solution treated with 7.60g (31.6mmoles) sodium sulfide nonahydrate. The mixture is refluxed overnight. The mixture is cooled and filtered to remove the black precipitate. The filtrate is evaporated to an oil, slurried in dichloromethane and treated with MgSO_4 . The mixture is filtered to remove the drying agent and the filtrate evaporated to leave 1,5,8,12,15-pentazabicyclo[10.5.2]nonadecane as an oil. Yield 4.80g (62%). Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental analysis.

To a solution containing 4.00g (14.8mmoles) 1,5,8,12,15-pentazabicyclo[10.5.2]nonadecane, 5.20g (48.4mmoles) Na_2CO_3 in 50mL 1,2-dimethoxyethane is added, dropwise, a solution containing 7.05mL (48.8mmoles, 10.2g) benzylbromoacetate in 25mL 1,2-dimethoxyethane. When the addition is complete, the mixture is heated briefly to reflux, and allowed to cool to room temperature, stirring overnight. The mixture is evaporated, slurried in 25mL dichloromethane, filtered to remove the salts present and purified by flash column chromatography (1x10cm, 50:50 ethyl acetate:hexanes, applied as CH_2Cl_2 solution). The appropriate fractions are combined and the solution filtered to remove any particulates. The filtrate is evaporated in vacuo leaving 5,8,15-tris(benzylacetato)-1,5,8,12,15-pentaazabicyclo[10.5.2]nonadecane as a pale oil. Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental analysis.

A slurry consisting of 1g 5% Pd on C and 4.00g (5.60mmoles) 5,8,15-tris(benzylacetato)-1,5,8,12,15-pentaazabicyclo[10.5.2]nonadecane in ethanol (95%) is shaken at 60psi H_2

overnight. The catalyst is removed by filtration and the filtrate evaporated to leave 5,8,15-tris(carboxymethyl)-1,5,8,12,15-pentaazabicyclo[10.5.2]nonadecane as a pale oil. Yield 2.21g (95%). Identity and purity of the product is confirmed by ¹H and ¹³C nmr, and elemental analysis.

Example 2

Synthesis of gadolinium(III) aqua-5,8,15-tris(carboxymethyl)-1,5,8,12,15-pentaaza-bicyclo[10.5.2]nonadecane.

A slurry containing 2.00g (4.81mmoles) 5,8,15-tris(carboxymethyl)-1,5,8,12,15-pentaazabicyclo[10.5.2]nonadecane and 1.74g (4.81mmoles) gadolinium oxide in 100mL water is refluxed until the mixture is clarified. Water is removed by evaporation and the residue dissolved in a mixture of boiling acetonitrile: absolute ethanol:iso-propyl alcohol 3:3:4, filtered hot and allowed to stand. Upon cooling crystals of gadolinium(III) aqua-5,8,15-tris(carboxymethyl)-1,5,8,12,15-pentaaza-bicyclo[10.5.2]nonadecane are deposited. Identity and purity of the product is confirmed by hplc examination and elemental analysis.

Example 3

Synthesis of 4,7,13-tris(carboxymethyl)-1,4,7,10,13-pentazabicyclo-[8.5.2]hepta-decane

In a 250mL round-bottom flask is placed 12.0g (42.3mmoles) 1-p-toluenesulfonyl-1,4,7-triazacyclononane, 100mL acetonitrile and 18.3g (92.8mmoles) N-tosylaziridine. The mixture is allowed to stir for three hours during which time the reaction may be monitored by thin layer chromatography (tlc). When the reaction is complete, acetonitrile is removed by evaporation

and the residue dissolved in dichloromethane. The solution is eluted through a 5x35cm column containing 500g silica gel. The chromatography is completed by elution with 2% methanol in dichloromethane. The fractions are checked by tlc, and
5 appropriately combined. The product, 1,4-bis(2-(p-toluenesulfonamido)ethyl)-7-(p-toluenesulfonyl)-1,4,7-triazacyclononane, may be isolated by evaporation of solvent as a white powdery solid. Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental analysis.

10

A mixture containing 22.0g (33.9mmoles) 1,4-bis(2-(p-toluenesulfonamido)ethyl)-7-(p-toluenesulfonyl)-1,4,7-triazacyclononane and 50mL concentrated sulfuric acid is allowed to stir overnight. The mixture is cooled to 0C and
15 poured carefully into 500mL dry, cold diethyl ether. The white solid which forms is collected by filtration and washed with cold ether. If the precipitate is tacky, or hygroscopic, the mother liquor of the diethyl ether-sulfuric acid slurry may be decanted, leaving the tacky residue. Treatment of the
20 precipitate with a solution of 6.5g sodium hydroxide in 200mL methanol followed by evaporation of solvent leaves a white precipitate. The solid is slurried with 200mL dichloromethane and treated with magnesium sulfate. After removing the drying agent by filtration, the solvent is removed by evaporation
25 leaving 1,4-bis(2-aminoethyl)-1,4,7-triazacyclononane as a clear, colorless oil. Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental analysis.

To a solution containing 6.00g (27.9mmoles) 1,4-bis(2-aminoethyl)-1,4,7-triazacyclononane in 95% ethanol is added
30 5.22g (30.7mmoles) copper (II) chloride dihydrate. The mixture is heated to reflux for two hours, then allowed to cool. Then solvent is removed by evaporation in vacuo and the residue redissolved in 95% aqueous methanol. To this solution
35 is added 6.33g (37.4mmoles) 40% aqueous glyoxal. The mixture

is refluxed overnight. After removing the solvent by evaporation, the residue is dissolved in 1L 90% aqueous methanol and treated with 3.26g (86.3mmoles) NaBH₄ and refluxed for one hour. After cooling to room temperature the mixture is evaporated to dryness, redissolved in 200mL fresh methanol and evaporated again. The residue is dissolved in 100mL water and the solution treated with 7.60g (31.6mmoles) sodium sulfite nonahydrate. The mixture is refluxed overnight. The mixture is cooled and filtered to remove the black precipitate. The filtrate is evaporated to an oil, slurried in dichloromethane and treated with MgSO₄. The mixture is filtered to remove the drying agent and the filtrate evaporated to leave 1,4,7,10,13-pentazabicyclo[8.5.2]heptadecane as an oil. Yield 3.70g (55%). Identity and purity of the product is confirmed by ¹H and ¹³C nmr, and elemental analysis.

To a solution containing 3.50g (14.5mmoles) 1,4,7,10,13-pentazabicyclo[8.5.2]heptadecane, 5.07g (47.9mmoles) sodium carbonate in 50mL 1,2-dimethoxyethane is added, dropwise, a solution containing 7.54mL (47.9mmoles, 10.9g) benzylbromoacetate in 25mL 1,2-dimethoxyethane. When the addition is complete, the mixture is heated briefly to reflux, and allowed to cool to room temperature, stirring overnight. The mixture is evaporated, slurried in 25mL dichloromethane, filtered to remove the salts present and purified by flash column chromatography (1x10cm, 50:50 ethyl acetate:hexanes, applied as CH₂Cl₂ solution). The appropriate fractions are combined and the solution filtered to remove any particulates. The filtrate is evaporated in vacuo leaving 4,7,13-tris(benzylacetato)-1,4,7,10,13-pentazabicyclo[8.5.2]heptadecane as a pale oil. Identity and purity of the product is confirmed by ¹H and ¹³C nmr, and elemental analysis.

A slurry consisting of 1g 5% Pd on C and 6.50g (9.48mmoles)

4,7,13-tris(benzylacetato)-1,4,7,10,13-pentazabicyclo-
[8.5.2]heptadecane in ethanol (95%) is shaken at 60psi H₂
overnight. The catalyst is removed by filtration and the
filtrate evaporated to leave 4,7,13-tris(carboxymethyl)-
1,4,7,10,13-pentazabicyclo[8.5.2]hepta-decane as a pale oil.
Identity and purity of the product is confirmed by ¹H and ¹³C
nmr, and elemental analysis.

Example 4

Synthesis of gadolinium(III) aqua-4,7,13-tris(carboxymethyl)-
1,4,7,10,13-pentaaza-bicyclo-[8.5.2]heptadecane

A slurry containing 3.50g (8.42mmoles) 4,7,13-
tris(carboxymethyl)-1,4,7,10,13-pentaaza-bicyclo-
[8.5.2]heptadecane and 1.50g (4.14mmoles) gadolinium oxide in
100mL water is refluxed until the mixture is clarified. Water
is removed by evaporation and the residue dissolved in a
mixture of boiling acetonitrile: absolute ethanol: iso-propyl
alcohol 3:3:4, filtered hot and allowed to stand. Upon
cooling crystals of gadolinium(III) aqua-4,7,13-
tris(carboxymethyl)-1,4,7,10,13-pentaaza-bicyclo-
[8.5.2]heptadecane are deposited. Identity and purity of the
product is confirmed by hplc examination and elemental
analysis.

Example 5

Synthesis of 4,7,12-tris(carboxymethyl)-12-methyl-1,4,7,10-
tetraaza-bicyclo[8.3.2]pentadecane

To a cold, 0C, solution containing 50.0g (416mmoles) 1,1,1-
tris(hydroxymethyl)ethane, and 1.00g (5.28mmoles) p-
toluenesulfonic acid in 300mL dry thf is added, dropwise,

33.6mL (26.6g, 458mmoles) acetone. When the addition is complete, the mixture is allowed to warm to room temperature, then refluxed for three hours. Solvent is removed in vacuo and the residue dissolved in 200mL CH_2Cl_2 . The solution is washed with 3x100mL 0.05N NaOH and the organic layer collected. After drying the solution with MgSO_4 , the mixture is filtered and solvent removed by evaporation, leaving 1-hydroxymethyl-1,4,4-trimethyl-3,5-dioxocyclohexane as a clear, colorless oil which crystallizes on standing. The solid is recrystallized from a minimum amount of boiling diethyl ether. Yield 63.3g (95%). Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental analysis.

A flask is charged with 60.0g (375mmoles) 1-hydroxymethyl-1,4,4-trimethyl-3,5-dioxocyclohexane, 127.5g (411mmoles) triphenylphosphite, and 35mL (79.8g, 562mmoles) iodomethane. The mixture is heated under gentle reflux until the temperature of the refluxing liquid rises from ca. 75C to ca. 130C. Volatiles are removed from the mixture by evaporation in vacuo and the residue is taken up in diethyl ether. The dark solution is slurried with 35g charcoal and refluxed on a steam bath. The mixture is filtered and solvent removed in vacuo. The resulting amber oil is purified by Kugelrohr distillation to give 1-iodomethyl-1,4,4-trimethyl-3,5-dioxocyclohexane. Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental analysis.

To a slurry consisting of 4.90g (202mmoles) magnesium turnings in 100mL diethylether is added dropwise a solution of 50g (185mmoles) 1-iodomethyl-1,4,4-trimethyl-3,5-dioxocyclohexane. The addition is at such a rate that a gentle reflux of ether is maintained. When the addition is complete, the mixture is refluxed an additional hour. The mixture is filtered to removed leftover magnesium and the filtrate cooled to 0C. A slow stream of dry (CaSO_4/KOH) CO_2 is bubbled into the solution

with stirring. After two hours the solution is warmed and gently refluxed. The mixture is cooled to 0C and 220mL 1.0N HCl in diethylether is added slowly. The mixture is filtered cold and the filtrate evaporated to give 1-carboxymethyl-1,4,4-trimethyl-3,5-dioxocyclohexane as a white solid. The solid is dissolved in 100mL boiling ethyl acetate and treated with hexanes to induce crystallization. Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental analysis.

To a solution containing 25.0g (133mmoles) 1-carboxymethyl-1,4,4-trimethyl-3,5-dioxocyclohexane and 15.2mL (15.8g, 146mmoles) benzyl alcohol in 400mL ethyl acetate is added 30.1g (146mmoles) DCC. The mixture is stirred at room temperature overnight. The large amount of precipitate which forms is removed by filtration. The clear colorless solution is washed with 1.0N HCl (3x200mL) and the organic layer collected. The solution is dried with MgSO_4 . After filtering to remove the drying agent, and any DCU which may precipitate, the solution is concentrated and treated with hexanes to induce crystallization to give 1-(benzylcarboxymethyl)-1,1-bis(hydroxymethyl)ethane. Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental analysis.

To a cold, 0C, solution of 20g (83.9mmoles) 1-(benzylcarboxymethyl)-1,1-bis(hydroxymethyl)ethane, and 26.0mL (18.7g, 185mmoles) NEt_3 in 200mL dichloromethane is added a solution of 35.3g (185mmoles) p-toluenesulfonyl chloride in 150mL dichloromethane. When the addition is complete the mixture is placed in a 0C refrigerator overnight. The large mass of crystals which form are removed by filtration. The filtrate is collected and reduced in volume in vacuo. The resulting oil is dissolved in fresh dichloromethane and washed with cold 2x150mL 0.1N HCl followed by cold brine. The organic layer is collected and treated with MgSO_4 . After the

drying agent is removed by filtration the filtrate is dissolved in a minimum of boiling ethyl acetate and treated with methanol to effect crystallization to afford 1-(benzylcarboxymethyl)-1,1-bis(tosyloxymethyl)ethane. Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental analysis.

A slurry consisting of 22.7g (164mmoles) K_2CO_3 and 26.1g (67.2mmoles) 1,4-bis(trimethethylsilylethylsulfonyl)-1,4-diazabutane in 150mL dry dmf is heated to 60C. To this is slowly added a solution of 40g (74.7mmoles) 1-(benzylcarboxymethyl)-1,1-bis(tosyloxymethyl)ethane in 200mL dry dmf. When the addition is complete the mixture is allowed to stir an additional 24 hours, with heat. After cooling the mixture to room temperature, the volatiles are removed by rotary-evaporation. The residue is shaken with 500g ice and the mixture filtered. The solid collected is wash with distilled water until the filtrate is neutral pH. The solid is dried on the filter with suction. The solid is dissolved in a minimum of boiling methanol, filtered hot and allowed to cool to effect crystallization of 1,5-bis(2-trimethylsilylethylsulfonyl)-3-(benzylcarboxymethyl)-3-methyl-1,5-diazacycloheptane. Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental analysis.

A slurry consisting of 20.0g (33.8mmoles) 1,5-bis(2-trimethylsilylethylsulfonyl)-3-(benzylcarboxymethyl)-3-methyl-1,5-diazacycloheptane and 15.5g (102mmoles) CsF in 100mL dry dmf is refluxed overnight. After cooling to room temperature 50mL methanol is added and the mixture evaporated in vacuo. The residue is diluted with 50mL diethyl ether, heated briefly to reflux, filtered and the filtrate evaporated. The residue is dissolved in 100mL fresh dry dmf and heated to 45C. To this solution is added 15.4g (74.4mmoles) 1-(2-trimethylsilylethylsulfonyl)aziridine in 100mL dry dmf. When

the addition is complete the mixture is heated to 70C overnight. After cooling the mixture to room temperature the solvent is removed in vacuo and the residue dissolved in a minimum of boiling methanol. The mixture is filtered hot and
5 allowed to cool slowly to effect crystallization of 1,5-bis(2-(2-trimethylsilylethylsulfonamido)ethyl)-3-(benzylcarboxymethyl)-3-methyl-1,5-diazacycloheptane. Identity and purity of the product is confirmed by ¹H and ¹³C nmr, and elemental analysis.

10

A slurry consisting of 10.0g (14.7mmoles) 1,5-bis(2-(2-trimethylsilylethylsulfonamido)ethyl)-3-(benzylcarboxymethyl)-3-methyl-1,5-diazacycloheptane and 6.70g (44.1mmoles) CsF in 100mL dry dmf is refluxed overnight. After cooling to room
15 temperature 50mL methanol is added and the mixture evaporated in vacuo. The residue is diluted with 50mL diethyl ether, heated briefly to reflux, filtered and the filtrate evaporated. The residue is dissolved in 100mL 95% ethanol and 3.80g (16.0mmoles) nickel(II) chloride hexahydrate is added.

20

The mixture is heated to reflux for two hours, then allowed to cool. Then solvent is removed by evaporation in vacuo and the residue redissolved in 95% aqueous methanol. To this solution is added 2.32g (16.0mmoles) 40% aqueous glyoxal. The mixture is refluxed overnight. After removing the solvent by

25

evaporation, the residue is dissolved in 500mL 90% aqueous methanol and treated with 1.20g (31.7mmoles) NaBH₄ and refluxed for one hour. After cooling to room temperature the mixture is evaporated to dryness, redissolved in 200mL fresh ethanol and concentrated by evaporation until the solution just

30

becomes turbid. A few drops of water are added until the mixture clarifies and the solution is allowed to cool slowly to room temperature. Further cooling effects crystallization of 12-(benzylcarboxymethyl)-12-methyl-1,4,7,10-tetrazabicyclo[8.3.2]pentadecane nickel(II) dichloride.

35

Identity and purity of the product is confirmed by hplc

examination and elemental analysis.

A solution of 3.00g (5.95mmoles) 12-(benzylcarboxymethyl)-12-methyl-1,4,7,10-tetrazabicyclo[8.3.2]pentadecane nickel(II) dichloride in 100mL water is treated with 1.60g (6.66mmoles) $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$. The mixture is refluxed overnight. The mixture is cooled and filtered to remove the black precipitate. The filtrate is evaporated to an oil, slurried in dichloromethane and treated with MgSO_4 . The mixture is filtered to remove the drying agent and the filtrate evaporated to leave 12-(benzylcarboxymethyl)-12-methyl-1,4,7,10-tetraazabicyclo[8.3.2]pentadecane as an oil. Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental analysis.

To a slurry containing 2.00g (5.34mmoles) 12-(benzylcarboxymethyl)-12-methyl-1,4,7,10-tetraazabicyclo[8.3.2]pentadecane and 1.30g (12.3mmoles) Na_2CO_3 in 50mL 1,2-dimethoxyethane is added, dropwise, a solution containing 1.90mL (12.0mmoles, 2.75g) benzylbromoacetate in 25mL 1,2-dimethoxyethane. When the addition is complete, the mixture is heated briefly to reflux, and allowed to cool to room temperature, stirring overnight. The mixture is evaporated, slurried in 10mL dichloromethane, filtered to remove the salts present and purified by flash column chromatography (1x10cm, 50:50 ethyl acetate:hexanes, applied as CH_2Cl_2 solution). The appropriate fractions are combined and the solution filtered to remove any particulates. The filtrate is evaporated in vacuo leaving a pale oil. The oil is dissolved in ethanol (95%) and shaken with 1g 5% Pd on C at 60psi H_2 overnight. The catalyst is removed by filtration and the filtrate evaporated to leave 4,7,12-tris(carboxymethyl)-12-methyl-1,4,7,10-tetraazabicyclo[8.3.2]pentadecane as a pale oil. Identity and purity of the product is confirmed by ^1H and ^{13}C nmr, and elemental

analysis.

Example 6

- 5 Synthesis of gadolinium(III) aqua-4,7,12-tris(acetato)-12-methyl-1,4,7,10-tetraaza-bicyclo[8.3.2]pentadecane

10 A slurry containing 1.00g (2.50mmoles) 4,7,12-tris(carboxymethyl)-12-methyl-1,4,7,10-tetraaza-bicyclo[8.3.2]pentadecane and 0.50g (1.38mmoles) gadolinium oxide in 100mL water is refluxed until the mixture is clarified. Water is removed by evaporation and the residue dissolved in a mixture of boiling acetonitrile: absolute
15 ethanol:iso-propyl alcohol 3:3:4, filtered hot and allowed to stand. Upon cooling crystals of gadolinium(III) aqua-4,7,12-tris(acetato)-12-methyl-1,4,7,10-tetraaza-bicyclo[8.3.2]pentadecane are deposited. Identity and purity of the product is confirmed by hplc examination and elemental
20 analysis.

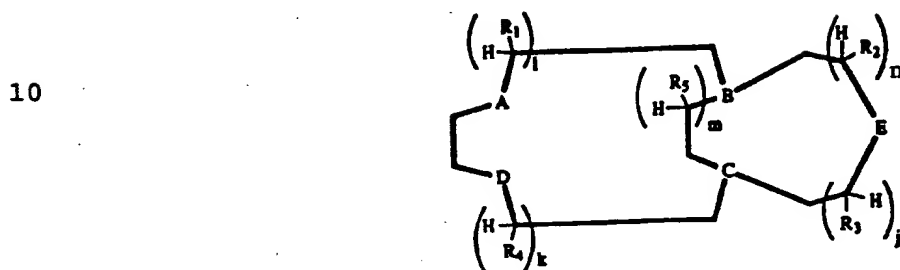
25 Although the invention has been described with respect to specific modifications, the details thereof are not to be construed as limitations, for it will be apparent that various equivalents, changes and modifications may be resorted to without departing from the spirit and scope thereof, and it is understood that such equivalent embodiments are to be included therein.

30

CLAIMS

5 What is claimed is:

1. A compound of the general formula:



Wherein A is N-G or P-G; B is N or P; C is N or P; D is N-G or P-G; E is N-G, P-G or C(R₆)-[CH(R₇)]_q-X; G is -[CH(R₈)]_i-X; X is -CO₂H, -OPO₃H₂, -PO₃H₂, -SO₃H, -SH, -OH, or -CONHOH; R₁, R₂, R₃, R₄, R₅, R₆, R₇ and R₈ can be the same or different and are hydrogen, C₁-C₈ alkyl, or C₆-C₁₀ aryl, optionally substituted by one or more hydroxy, C₁-C₈ alkyl, C₁-C₈ hydroxyalkyl, C₁-C₈ alkoxy, C₆-C₁₀ aryl, C₆-C₁₀ hydroxyaryl, C₆-C₁₀ aryloxy, -CO₂R₉, -CONR₁₀R₁₁, or -NR₁₀R₁₁ groups; R₉, R₁₀, and R₁₁ may be the same or different and are selected from the group consisting of hydrogen, C₁-C₈ alkyl, C₁-C₈ hydroxyalkyl and C₁-C₈ alkoxyalkyl; R₁₀ and R₁₁ may form a 5 or 6 membered carbocyclic ring optionally containing singularly or in combination nitrogen, oxygen or sulfur; i, j, k, l, m, n and q may be the same or different and are zero to about 5.

30

2. The compound of claim 1 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is -[CH(R₈)]_i-X; X is CO₂H; R₁ is H; R₂ is H; R₃ is H; R₄ is H; R₅ is H; R₆ is H; i is 1; j is 1; k is 1; l is 1, m is 1; and n is 1.

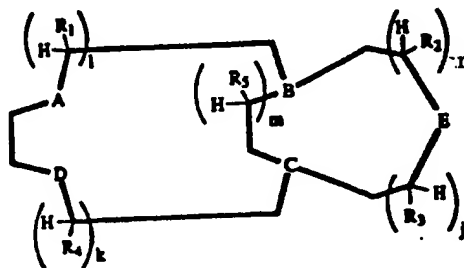
35

3. The compound of claim 1 wherein A is N-G; D is N-G; B is N; C is N; E is $-C(R_6)-[CH(R_7)]_q-X$; G is $-[CH(R_8)]_l-X$; X is $-CO_2H$; R_6 is $-CH_3$, R_7 is H; R_8 is H; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; i is 1; k is 1; l is 1; m is 1; q is 1; n is 0; and j is 0.

4. The compound of claim 1 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is $-[CH(R_8)]_l-X$; X is $-CO_2H$; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; R_6 is H; i is 1; j is 1; m is 1; n is 1; l is 2; and k is 2.

5. The compound of claim 1 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is $-[CH(R_8)]_l-X$; X is $-SH$; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; R_6 is H; j is 1; k is 1; l is 1, m is 1; n is 1; and i is 2.

6. A compound of the general formula:



Wherein A is N-G or P-G; B is N or P; C is N or P; D is N-G or P-E; E is N-G, P-G or $C(R_6)-[CH(R_7)]_q-Y$; G is $-[CH(R_8)]_l-Y$; Y is $-CO_2M$, $-OPO_3HM$, $-PO_3HM$, $-SO_3M$, $-SM$, $-OM$, or $-CONHOM$:

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 can be the same or different and are hydrogen, C_1-C_8 alkyl, or C_6-C_8 aryl, optionally substituted by one or more hydroxy, C_1-C_8 alkoxy, C_1-C_8 hydroxyalkyl, C_1-C_8 alkoxy, C_6-C_{10} hydroxyaryl, C_6-C_{10}

aryloxy, $-CO_2R_9$, $-CONR_{10}R_{11}$, or $NR_{10}R_{11}$ groups, R_9 , R_{10} , and R_{11} may

be the same or different and are selected from the group consisting of hydrogen, C₁-C₈ alkyl alkyl, C₁-C₈ hydroxyalkyl and C₁-C₈ alkoxyalkyl; R₁₀ and R₁₁ may form a 5 or 6 membered carbocyclic ring optionally containing singularly or in
5 combination nitrogen, oxygen or sulfur; i, j, k, l, m, n and q may be the same or different and are zero to about 5; and M is a metal ion equivalent and/or a physiologically acceptable cation of an organic base.

10 7. The compound of claim 6 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is -[CH(R₈)]_i-Y; Y is CO₂M; R₁ is H; R₂ is H; R₃ is H; R₄ is H; R₅ is H; R₆ is H; i is 1; j is 1; k is 1; l is 1; m is 1; n is 1; and M is gadolinium.

15 8. The compound of claim 6 wherein A is N-G; D is N-G; B is N; C is N; E is C(R₆)-[CH(R₇)]_q-Y; G is [CH(R₈)]_i-Y; Y is -CO₂M; R₁ is H; R₂ is H; R₃ is H; R₄ is H; R₅ is H; R₆ is -CH₃; R₇ is H; R₈ is H; i is 1; k is 1; l is 1; m is 1; q is 1; n is 0; j is 0; and M is dysprosium.

20 9. The compound of claim 6 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is -[CH(R₈)]_i-Y; Y is -CO₂M; R₁ is H; R₂ is H; R₃ is H; R₄ is H; R₅ is H; R₆ is H; i is 1; j is 1; m is 1; n is 1; l is 2; k is 2; and M is gadolinium.

25 10. The compound of claim 6 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is -[CH(R₈)]_i-Y; Y is SM; R₁ is H; R₂ is H; R₃ is H; R₄ is H; R₅ is H; R₆ is H; j is 1; k is 1; l is 1; m is 1; n is 1; i is 2; and M is iron.

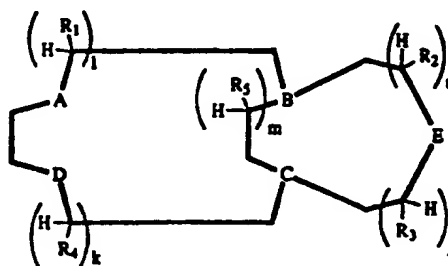
30 11. The compound of claim 6 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is -[CH(R₈)]_i-Y; Y is -CO₂M; R₁ is H; R₂ is H; R₃ is H; R₄ is H; R₅ is H; R₆ is H; i is 1; j is 1; k is 1; l is 1; m is 1; n is 1; and M is yttrium.

35

12. The compound of claim 6 wherein A is N-G; D is N-G; B is N; C is N; E is $-C(R_6)-[CH(R_7)]_q-Y$; G is $[CH(R_8)]_1-Y$; Y is CO_2M ; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; R_7 is H; R_6 is $-CH_3$; R_8 is H; i is 1; k is 1; l is 1; m is 1; q is 1; n is 0; j is 0; and M is indium.
13. The compound of claim 6 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is $-[CH(R_8)]_1-Y$; Y is CO_2M ; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; R_8 is H; i is 1; j is 1; m is 1; n is 1; l is 2; k is 2; and M is gallium.
14. The compound of claim 6 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is $-[CH(R_8)]_1-Y$; Y is SM; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; R_8 is H; j is 1; k is 1; l is 1; m is 1; n is 1; i is 2; and M is indium.
15. The compound of claim 6 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is $-[CH(R_8)]_1-Y$; Y is $-CO_2M$; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; R_8 is H; i is 1; j is 1; k is 1; l is 1; m is 1; n is 1; and M is holmium.
16. The compound of claim 6 wherein A is N-G; D is N-G; B is N; C is N; E is $-C(R_6)-[CH(R_7)]_q-Y$; G is $-[CH(R_8)]_1-Y$; Y is $-CO_2M$; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; R_7 is H; R_6 is $-CH_3$; R_8 is H; i is 1; k is 1; l is 1; m is 1; n is 0; j is 0; and M is gadolinium.
17. The compound of claim 6 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is $-[CH(R_8)]_1-Y$; Y is $-CO_2M$; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; R_8 is H; i is 1; j is 1; m is 1; n is 1; l is 2; k is 2; and M is dysprosium.
18. The compound of claim 6 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is $-[CH(R_8)]_1-Y$; Y is SM; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; R_8 is H; j is 1; k is 1; l is 1; m

is 1; n is 1; i is 2; and M is bismuth.

19. A method of imaging comprising administering to a patient
an imaging enhancing amount of a compound of the general
5 formula:



Wherein A is N-G or P-G; B is N or P; C is N or P; D is
N-G or P-G; E is N-G, P-G or $C(R_6)-[CH(R_7)]_q-Y$; G is $-[CH(R_8)]_1-$
Y; Y is $-CO_2M$, $-OPO_3HM$, $-PO_3HM$, $-SO_3M$, $-SM$, $-OM$, or $-CONHOM$; R_1 ,
 R_2 , R_3 ,

R_4 , R_5 , R_6 , R_7 , and R_8 can be the same or different and are
hydrogen, C_1-C_8 alkyl, or C_6-C_8 aryl, optionally
substituted by one or more hydroxy, C_1-C_8 alkoxy, C_1-C_8
hydroxyalkyl, C_1-C_8 alkoxy, C_6-C_{10} hydroxyaryl, C_6-C_{10} aryloxy, $-$
 CO_2R_9 , $-CONR_{10}R_{11}$, or $NR_{10}-R_{11}$ groups, R_9 , R_{10} , and R_{11} may be the
same or different and are selected from the group consisting
of hydrogen, C_1-C_8 alkyl, C_1-C_8 hydroxyalkyl and C_1-C_8
alkoxyalkyl; R_{10} and R_{11} may form a 5 or 6 membered carbocyclic
ring optionally containing singularly or in combination
nitrogen, oxygen or sulfur; i, j, k, l, m, n and q may be the
same or different and are zero to about 5; and M is a metal
ion equivalent and/or a physiologically acceptable cation of
an organic base.

20. The method of claim 19 wherein A is N-G; D is N-G; E is
N-G; B is N; C is N; G is $-[CH(R_8)]_1-Y$; Y is CO_2M ; R_1 is H; R_2

is H; R_3 is H; R_4 is H; R_5 is H; R_6 is H; i is 1; j is 1; k is 1; l is 1; m is 1; n is 1; and M is gadolinium.

21. The method of claim 19 wherein A is N-G; D is N-G; B is N; C is N; E is $C(R_6)-[CH(R_7)]_q-Y$; G is $[CH(R_8)]_i-Y$; Y is $-CO_2M$; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; R_6 is $-CH_3$; R_7 is H; R_8 is H; i is 1; k is 1; l is 1; m is 1; q is 1; n is 0; j is 0; and M is dysprosium.

22. The method of claim 19 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is $-[CH(R_8)]_i-Y$; Y is $-CO_2M$; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; R_6 is H; i is 1; j is 1; m is 1; n is 1; l is 2; k is 2; and M is gadolinium.

23. The method of claim 19 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is $-[CH(R_8)]_i-Y$; Y is SM; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; R_6 is H; j is 1; k is 1; l is 1; m is 1; n is 1; i is 2; and M is iron.

24. The method of claim 19 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is $-[CH(R_8)]_i-Y$; Y is $-CO_2M$; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; R_6 is H; i is 1; j is 1; k is 1; l is 1; m is 1; n is 1; and M is yttrium.

25. The method of claim 19 wherein A is N-G; D is N-G; B is N; C is N; E is $-C(R_6)-[CH(R_7)]_q-Y$; G is $[CH(R_8)]_i-Y$; Y is CO_2M ; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; R_7 is H; R_6 is $-CH_3$; R_8 is H; i is 1; k is 1; l is 1; m is 1; q is 1; n is 0; j is 0; and M is indium.

26. The method of claim 19 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is $-[CH(R_8)]_i-Y$; Y is CO_2M ; R_1 is H; R_2 is H; R_3 is H; R_4 is H; R_5 is H; R_6 is H; i is 1; j is 1; m is 1; n is 1; l is 2; k is 2; and M is gallium.

27. The method of claim 19 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is $-\text{[CH(R}_8\text{)]}_1\text{-Y}$; Y is SM; R₁ is H; R₂ is H; R₃ is H; R₄ is H; R₅ is H; R₆ is H; j is 1; k is 1; l is 1; m is 1; n is 1; i is 2; and M is indium.

5

28. The method of claim 19 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is $-\text{[CH(R}_8\text{)]}_1\text{-Y}$; Y is $-\text{CO}_2\text{M}$; R₁ is H; R₂ is H; R₃ is H; R₄ is H; R₅ is H; R₆ is H; i is 1; j is 1; k is 1; l is 1; m is 1; n is 1; and M is holmium.

10

29. The method of claim 19 wherein A is N-G; D is N-G; B is N; C is N; E is $-\text{C(R}_6\text{)-[CH(R}_7\text{)]}_q\text{-Y}$; G is $-\text{[CH(R}_8\text{)]}_1\text{-Y}$; Y is $-\text{CO}_2\text{M}$; R₁ is H; R₂ is H; R₃ is H; R₄ is H; R₅ is H; R₇ is H; R₆ is $-\text{CH}_3$; R₈ is H; i is 1; k is 1; l is 1; m is 1; n is 0; j is 0; and M is gadolinium.

15

30. The method of claim 19 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is $-\text{[CH(R}_8\text{)]}_1\text{-Y}$; Y is $-\text{CO}_2\text{M}$; R₁ is H; R₂ is H; R₃ is H; R₄ is H; R₅ is H; R₆ is H; i is 1; j is 1; m is 1; n is 1; l is 2; k is 2; and M is dysprosium.

20

31. The method of claim 19 wherein A is N-G; D is N-G; E is N-G; B is N; C is N; G is $-\text{[CH(R}_8\text{)]}_1\text{-Y}$; Y is SM; R₁ is H; R₂ is H; R₃ is H; R₄ is H; R₅ is H; R₆ is H; j is 1; k is 1; l is 1; m is 1; n is 1; i is 2; and M is bismuth.

25

30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/00634

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 49/00; C07D 245/00, 487/00

US CL : 534/15, 16; 424/9.363; 540/472, 473

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 534/15, 16; 424/9.363; 540/472, 473

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN Structure Search - Files CA, Registry.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A, 91/10669 (NYCOMED) 25 JULY 1991, see pages 6-8, 10.	1-31
Y	US, A, 4,678,667 (MEARES et al.) 07 JULY 1987, see column 5.	1-31
Y	J. Am. Chem. Soc., Vol. 112, issued 1990, FORTIER et al., "Template Synthesis....," pages 2640-2647, see Scheme II.	1-31
Y	J. Chem. Soc. Comm., issued 1982, Ramasubbu et al., "Structurally Reinforced Cyclen....," pages 277-278, see scheme I.	1-31
Y	J. Am. Chem. Soc., Vol. 110, issued 1988, Hancock et al., "More Rigid Macrocyclic Ligands....," pages 2788-2794, see Figure 2.	1-31

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	* T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A		document defining the general state of the art which is not considered to be of particular relevance
* E	* X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* L		document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
* O	* Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* P	* &	document published prior to the international filing date but later than the priority date claimed
		document member of the same patent family

Date of the actual completion of the international search 24 APRIL 1995	Date of mailing of the international search report 18 MAY 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer L. A. CAMPBMAN sso Telephone No. (703) 308-1235

